ANESTHESIOLOGY

An Assessment of Penetrance and Clinical Expression of Malignant Hyperthermia in Individuals Carrying Diagnostic Ryanodine Receptor 1 Gene Mutations

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Malignant hyperthermia is a rare life-threatening disorder triggered in genetically predisposed individuals by exposure to certain anesthetics
- The ryanodine receptor 1 (*RYR1*) gene, which encodes the Ca2+ release channel of skeletal muscle sarcoplasmic reticulum, is the major malignant hyperthermia-associated locus
- Malignant hyperthermia diagnostic mutations are more prevalent than the reported incidence of clinical malignant hyperthermia episodes because many mutation carriers are never exposed to anesthetic triggers and some may have several uneventful anesthetics before developing malignant hyperthermia reaction

What This Article Tells Us That Is New

- In a multicenter case-control study of 229 genotype-positive subjects with previous recorded exposure to trigger anesthetics, there were 93 malignant hyperthermia cases, for an overall penetrance for the analyzed *RYR1* mutations of 40.6%
- The probability of developing malignant hyperthermia on exposure to triggers was 0.25 among all *RYR1* mutation carriers and 0.76 in survivors of malignant hyperthermia reactions (95% CI of the difference 0.41 to 0.59)
- Young age, male sex, and the use of succinylcholine were major nongenetic risk factors influencing expression of the *RYR1* mutations conferring malignant hyperthermia susceptibility

ABSTRACT

Background: Malignant hyperthermia (MH) is a potentially lethal disorder triggered by certain anesthetics. Mutations in the ryanodine receptor 1 (*RYR1*) gene account for about half of MH cases. Discordance between the low incidence of MH and a high prevalence of mutations has been attributed to incomplete penetrance, which has not been quantified yet. The authors aimed to examine penetrance of MH-diagnostic *RYR1* mutations and the likelihood of mutation carriers to develop MH, and to identify factors affecting severity of MH clinical expression.

Methods: In this multicenter case–control study, data from 125 MH pedigrees between 1994 and 2017 were collected from four European registries and one Canadian registry. Probands (survivors of MH reaction) and their relatives with at least one exposure to anesthetic triggers, carrying one diagnostic *RYR1* mutation, were included. Penetrance (percentage of probands among all genotype-positive) and the probability of a mutation carrier to develop MH were obtained. MH onset time and Clinical Grading Scale score were used to assess MH reaction severity.

Results: The overall penetrance of nine *RYR1* diagnostic mutations was 40.6% (93 of 229), without statistical differences among mutations. Likelihood to develop MH on exposure to triggers was 0.25 among all *RYR1* mutation carriers, and 0.76 in probands (95% CI of the difference 0.41 to 0.59). Penetrance in males was significantly higher than in females (50% [62 of 124] *vs.* 29.7% [30 of 101]; $P = 0.002$). Males had increased odds of developing MH (odds ratio, 2.37; 95% CI, 1.36 to 4.12) despite similar levels of exposure to trigger anesthetics. Proband's median age was 12 yr (interquartile range 6 to 32.5).

Conclusions: Nine MH-diagnostic *RYR1* mutations have sex-dependent incomplete penetrance, whereas MH clinical expression is influenced by patient's age and the type of anesthetic. Our quantitative evaluation of MH penetrance reinforces the notion that a previous uneventful anesthetic does not preclude the possibility of developing MH.

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Malignant hyperthermia (MH) is a rare life-threatening disorder caused by dysregulation of intracellular calcium homeostasis in skeletal muscle and triggered by exposure to certain anesthetics in genetically predisposed individuals.[1] A progressively better understanding of the pathomechanism of MH, advances in anesthesia monitoring, and the introduction of dantrolene have been crucial in reducing MH mortality, which remains around 10%.[2]

Variants in ryanodine receptor 1 (*RYR1*),[3] calcium voltage-gated channel subunit alpha1 S (*CACNA1S*),[4–6] and in SH3 and cysteine rich domain 3 (*STAC3*) genes[7] are associated with MH. The *RYR1* gene—encoding the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum (RyR1)—is the major MH-associated locus, involved in more than half of MH cases, whereas variants in *CACNA1S* and *STAC3* account for less than 1%. At present, 48 *RYR1* and 2 *CACNA1S* variants are

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recognized as MH-causative mutations.[8] Recent availability of data on thousands of human exomes[9] has allowed to determine the true combined prevalence of all known MH-causative mutations as 1:2,750,[10] which is close to earlier estimates.[11–13] The prevalence of MH diagnostic mutations is considerably greater than the reported incidence of clinical MH episodes (1:35,000 to 1:68,000 surgical discharges).[2] This striking discrepancy can be attributable to the fact that many mutation carriers may never be exposed to anesthetic triggers.[14] The discrepancy may also reflect a reduced—or incomplete—penetrance of the MH trait.[10,11,15] Indeed, not all subjects carrying a causative mutation develop MH on first exposure to anesthesia, and some may have several uneventful anesthetics before developing MH in the operating room.[16,17] In addition, the onset, progression, and severity of MH reaction are variable. The time of onset of MH seems to be influenced by the type and dose of volatile anesthetic, whereas severity is also dependent on the duration of exposure.[18–20]

Although our knowledge of factors influencing MH penetrance is limited, it is known that MH penetrance may depend on the additive effect of more than one genetic factor,[21] and allele-specific differences in RyR1 mRNA expression levels may explain the observed reduced penetrance and variations in MH phenotype among individuals.[22] Several studies[23–26] indicate higher incidence of MH in younger males, but the reason for that remains unclear.

Challenged by a paucity of clinically affected individuals with variable phenotype and known genotype, quantification of the MH trait penetrance remains an elusive subject, albeit being essential for an optimal risk assessment at the time of genetic counseling. In this study, we analyzed the available clinical and genetic data on 125 European and North American MH families collected at MH centers in Europe and Canada. We hypothesized that the penetrance of diagnostic *RYR1* mutations is incomplete, and that reduced penetrance and MH clinical expression are genotype-specific. Therefore, our objectives were to examine the penetrance of *RYR1* diagnostic mutations and the likelihood of *RYR1* mutation carriers to develop MH, and to identify factors that may affect MH clinical expression.

This article is featured in "This Month in Anesthesiology," page 1A. This article is accompanied by an editorial on p. 957. Part of the work presented in this article has been presented at the International Anesthesia Research Society Meeting (IARS) on April 28, 2018, in Chicago, Illinois. This article has a visual abstract available in the online version.

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Materials and Methods

After research ethics board approval and institutional authorization, for this multicenter case–control study, we collected clinical and genetic data on MH susceptible individuals from families with a positive history of MH reaction from the registries of three German, one Belgian, and one Canadian MH diagnostic centers between January 1, 1994 and December 31, 2017. Because of the retrospective nature of the study, consent was waived by the ethics board of all the participating centers.

Selection Process and Data Collection

Probands (survivors of MH reactions) and their relatives (family members who carried a familial *RYR1* mutation and had one or more uneventful exposures to anesthetic triggers) were included in the study provided that: (1) they carried only a single MH diagnostic *RYR1* mutation and had no additional potentially pathogenic *RYR1* variants; (2) data on two or more probands sharing a *RYR1* mutation were available; (3) three or more relatives (excluding the probands) shared a *RYR1* mutation and each had a documented history of at least one uneventful exposure to MH triggers (fig. 1).

RYR1 mutation refers to nonsynonymous variants that have been functionally validated and included in the MH diagnostic mutations list of the European Malignant Hyperthermia Group.[8] When referring to specific *RYR1* mutations we describe the inferred amino acid change at the protein level using the Human Genome Variation Society recommendations for the description of sequence variants.[27]

Data collected on probands included *RYR1* genotype, number of exposures (*i.e.,* number of surgeries under general anesthesia with inhalational agents or succinylcholine, including the one during which the MH crisis occurred), trigger agent(s) used, sex, age at the time of the MH reaction, Clinical Grading Scale[28] scores, and onset time defined as the period from the start of the trigger anesthetic to first sign of MH on record. Data were extracted from the anesthetic records, where available, or otherwise from the MH center records.

Available data on relatives of the probands included *RYR1* genotype, sex, and number of exposures (*i.e.*, uneventful anesthetics with trigger agents). The relatives' ages at the time of triggered anesthetics were not extracted because they were not available for all.

Penetrance[29] of an MH causative *RYR1* mutation was defined as the percentage of probands among all carriers of the mutation who had been exposed to general anesthesia:

$$Penetrance = \frac{n_{probands}}{\left(n_{relatives} + n_{probands}\right)} \times 100$$

$n_{probands}$ and $n_{relatives}$ refer to the number of probands and of relatives, respectively. Because of the rarity of MH, penetrance

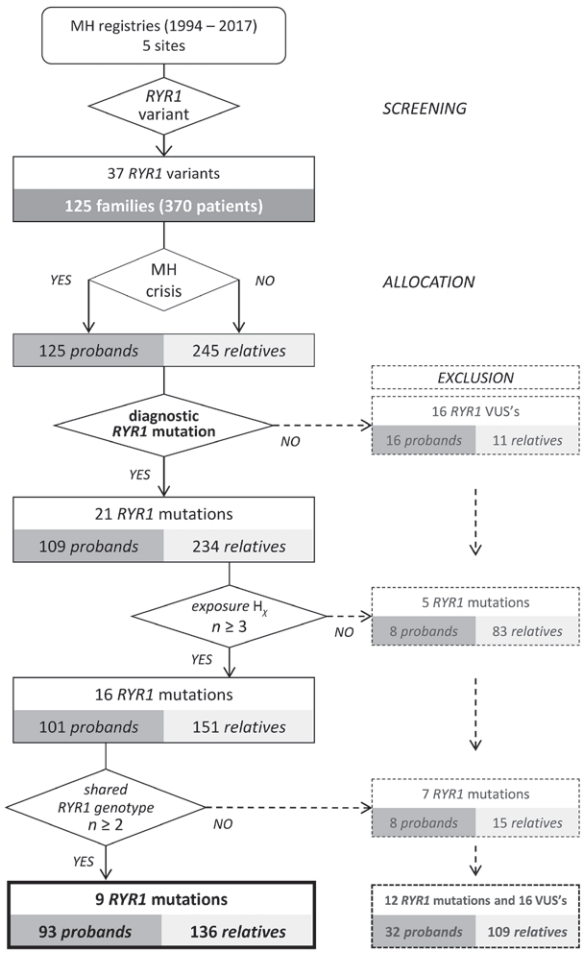

Fig. 1. The five diamonds on the flowchart contain the criteria used in this research for screening, allocation and inclusion/exclusion according to: (1) variant pathogenicity as per the list of diagnostic malignant hyperthermia (MH) mutations from the European Malignant Hyperthermia Group; (2) history (H_χ) of uneventful exposure to trigger anesthetics in relatives bearing a given mutation; (3) available data from at least two probands sharing a ryanodine receptor 1 (*RYR1*) mutation. VUSs, variants of unknown significance.

in this study is a byproduct of pooled pedigrees with a shared *RYR1* mutation and therefore it is subject to the influence of different familial genetic backgrounds.

MH susceptible individuals may have several uneventful exposures to triggering anesthetics before developing an MH reaction. We assessed the probability to develop MH on exposure to triggers in carriers of a *RYR1* mutation both in probands ($P_{\mathrm{MH}\ probands}$) and in the entire cohort ($P_{\mathrm{MH}\ all}$), respectively, as follows:

$$P_{\mathrm{MH}\ probands} = \frac{n_{probands}}{Exp_{probands}}$$

$$P_{\mathrm{MH}\ all} = \frac{n_{probands}}{\left(Exp_{probands} + Exp_{relatives}\right)}$$

Here, $n_{probands}$ is the number of probands, which in this study is equal to the number of MH reactions, and $Exp_{(x)}$ is the number of exposures to triggers in probands or in relatives, as specified.[30,31]

Clinical Grading Scale scores and MH onset time were used as indices of MH phenotype severity for comparison of the different *RYR1* genotypes.

Statistical Analysis

No statistical power calculation was conducted before the study, so the sample size was based on the available data. Hypothesis testing was two-tailed. Normality of the different variables was graphically assessed by histograms or by the Kolmogorov–Smirnov test for normality. Mean and SD were used to describe normally distributed data, whereas median and interquartile range were used for skewed variables. Chi-square test was used to look for associations between the phenotype groups (*probands* or *relatives*) and the *RYR1* genotype, the number of exposures to anesthetic triggers, and sex. Pairwise comparisons of penetrance and P_{MH} across the *RYR1* mutations were performed using *Z*-test with Bonferroni's correction to decrease the likelihood of committing type 1 error ($P < [\alpha / \binom{9}{2}] \rightarrow [0.05/36] = 0.0014$). Nonparametric between-subjects one-way ANOVA was used to assess differences in MH phenotype severity. Spearman's coefficient was used to explore the correlation between proband's age and each indicator of clinical MH severity (*i.e.*, Clinical Grading Scale score and MH onset time). Only this latter analysis was data-driven (*post hoc*), whereas all the former were done in accordance to our original statistical plan. $P < 0.05$ was considered significant, unless otherwise specified. The software used for statistical analysis was SAS Studio (Enterprise Edition, version 3.7; SAS Institute Inc., USA).

Results

An initial screening of the four European and one Canadian MH registries revealed 125 unrelated MH pedigrees with 370 individuals carrying 37 different potentially pathogenic *RYR1* variants. After applying the inclusion criteria (see Materials and Methods), 229 subjects from 93 MH pedigrees (93 probands and 136 relatives) carrying nine MH diagnostic mutations were included in this study (fig. 1). Among those excluded, there were eight families with more than one potentially pathogenic *RYR1* variant. Data were extracted from anesthetic records in 76 probands and from the referring anesthesiologists' reports in the rest of the study sample.

The selected *RYR1* mutation carriers had in total 365 exposures to anesthesia with MH triggers, of which 93

Table 1. Penetrance and Likelihood to Develop MH, by *RYR1* Genotype

RYR1 Mutation	Relatives		Probands		Penetrance (%)	MH Probability (P_{MH})	
	n	*#Exp*	*n*	*#Exp*		$P_{MH\ all}$	$P_{MH\ probands}$
p.Gly341Arg	18	25	11	17	37.9	0.26	0.65
p.Arg614Cys	33	64	30	39	47.6	0.29	0.77
p.Arg614Leu	3	5	5	6	**62.5**	**0.45**	0.83
p.Thr2206Met	36	57	18	19	33.3	0.24	**0.95**
p.Arg2336His	5	12	3	5	37.5	0.18	**0.60**
p.Ala2350Thr	4	9	3	4	42.9	0.23	0.75
p.Gly2375Ala	7	11	4	5	36.4	0.25	0.80
p.Gly2434Arg	9	17	12	18	57.1	0.34	0.67
p.Arg2454His	21	43	7	9	**25**	**0.13**	0.78
Overall	136	243	93	122	40.6 ± 0.12	0.25 ± 0.09*	0.76 ± 0.11
Exposures	2 [1–2]*		1 [1-1]				

Overall penetrance and P_{MH} expressed as mean ± SD; exposures expressed as median [interquartile range]. Penetrance = $n_{probands}$ / ($n_{relatives}$ + $n_{probands}$) × 100; $P_{MH\ probands}$ = $n_{probands}$ / $\#Exp_{probands}$; $P_{MH\ all}$ = $n_{probands}$ / ($\#Exp_{probands}$ + $\#Exp_{relatives}$). *#Exp*, number of exposures (*i.e.*, general anesthetics received with trigger agents); MH, malignant hyperthermia; *RYR1*, ryanodine receptor 1. Range (highest and lowest) values are showed in boldface, P = 0.047, 0.054 and 0.038 for penetrance, $P_{MH\ all}$ and $P_{MH\ probands}$ range values, respectively.

*P < 0.001.

resulted in MH reactions (table 1). The median exposure to MH triggers was higher in relatives than in probands (2 *vs.* 1 exposures per subject, $P < 0.0001$; table 1). Among probands, 79.6% (74 of 93) developed an MH reaction during the first anesthetic, 17.2% (16 of 93) during the second, and 3.2% (3 of 93) after more than two exposures to MH triggers (fig. 2).

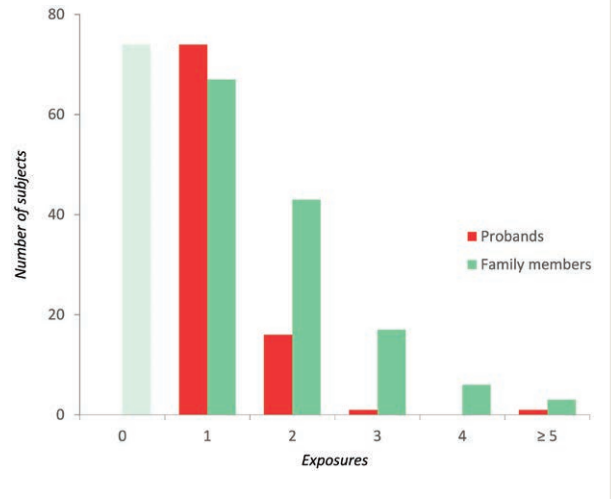

Fig. 2. Exposures to general anesthetics with trigger agents in carriers of ryanodine receptor 1 mutations. On the vertical and horizontal axes are represented the number of subjects exposed to trigger anesthetics and the total number of exposures, respectively. Exposures in probands (*red*) include the general anesthetic during which the actual malignant hyperthermia crisis occurred, whereas in family members (*green*) they comprise the total number of uneventful anesthetics with trigger agents; family members with no anesthetic history (*shaded green bar*) were excluded from analysis.

Penetrance

RYR1 Genotype. The MH-diagnostic *RYR1* mutations harbored by the study participants included three amino-terminal mutations (p.Gly341Arg, p.Arg614Cys, and p.Arg614Leu) and six mutations within the central RyR1 region (p.Thr2206Met, p.Arg2336His, p.Ala2350Thr, p.Gly2375Ala, p.Gly2434Arg, and p.Arg2454His).

There were no missing data for genotype, neither in probands nor in relatives. There were 93 MH cases among 229 genotype-positive subjects with previous recorded exposure to trigger anesthetics; that yields an overall penetrance for the analyzed *RYR1* mutations of 40.6% (95% CI, 34.3 to 47.3%). Notably, levels of penetrance were not significantly different (P = 0.303) among the analyzed *RYR1* mutations (table 1). Even the difference between mutations with the highest and lowest penetrance did not reach statistical significance (62.5% [5 of 8] for p.Arg614Leu, and 25% [7 of 28] for p.Arg2454His, respectively; P = 0.047, whereas $P < 0.0014$ was required after Bonferroni's correction for 36 pairwise comparisons).

The overall probability that a carrier of any of the nine *RYR1* diagnostic mutations will develop MH on exposure to triggers, $P_{MH\ all}$, was 0.25 (95% CI, 0.21 to 0.30). $P_{MH\ all}$ ranged from 0.45 to 0.13 for the same aforementioned pair of mutations (P = 0.054). However, if only probands were considered, the probability of developing MH ($P_{MH\ probands}$) increased to 0.76 (95% CI, 0.67 to 0.83; table 1).

Demographic Factors and MH Triggers. There was a significant association between sex and phenotype: 67.4% (62 of 92) probands were males, whereas among relatives males comprised 46.6% (62 of 133; P = 0.002; table 2). One proband and three relatives did not have data for sex and were excluded from the analysis.

Table 2. Sex Distribution by Phenotype Group and *RYR1* Genotype (Upper Section) and Penetrance, Likelihood to Develop MH, and Exposure Rate by Sex (Lower Section)

	Males		Females	
***RYR1* mutation**	**Proband**	**Relative**	**Proband**	**Relative**
p.Gly341Arg	8	6	3	11
p.Arg614Cys	18	15	12	18
p.Arg614Leu	3	2	2	1
p.Thr2206Met	11	19	6	17
p.Arg2336His	2	2	1	3
p.Ala2350Thr	3	2	0	2
p.Gly2375Ala	4	3	0	4
p.Gly2434Arg	9	4	3	5
p.Arg2454His	4	9	3	10
N	*62*	*62*	*30*	*71*
# Exposures	87	110	34	126
Penetrance	50 ± 0.11*		29.7 ± 0.18	
Exposures	1 [1-1]		1 [1-1]	
$P_{MH\text{-}all}$	0.31 ± 0.09		0.19 ± 0.12	
$P_{MH\text{-}probands}$	0.71 ± 0.16		0.88 ± 0.14	

Sex data were missing in one proband and three relatives. Penetrance and P_{MH} are expressed as mean ± SD; *Exposures* as median [interquartile range]. MH, malignant hyperthermia; *RYR1*, ryanodine receptor 1.

*$P < 0.01$.

The overall penetrance of the MH trait was significantly higher in males compared with females (50% [62 of 124] *vs.* 29.7% [30 of 101]; 95% CI of the difference 7 to 32%, $P = 0.002$]. Moreover, males had increased odds of developing MH compared with females (odds ratio, 2.37; 95% CI, 1.36 to 4.12) despite similar levels of exposure to trigger anesthetics for both sexes, with an overall rate of 1.6 exposures per subject (table 2).

Age distribution in probands was positively skewed (fig. 3), with a median age of 12 yr and an overwhelming majority being younger than 33 yr old (interquartile range 6 to 32.5; table 3). Age was missing for five probands, who were excluded from the MH phenotype severity analysis.

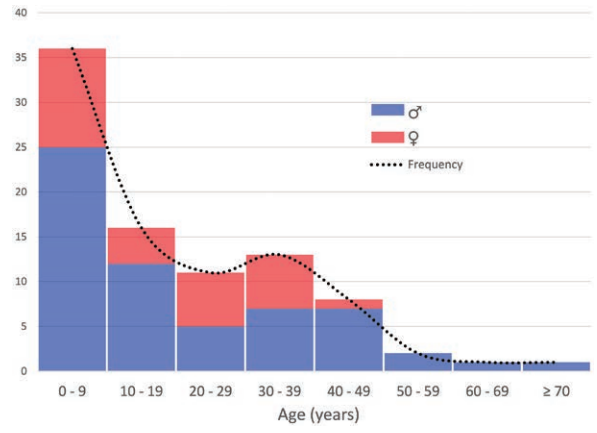

Fig. 3. Age distribution at the time of malignant hyperthermia (MH) crisis by sex, in MH probands carrying ryanodine receptor 1 mutations.

There were no missing data regarding the anesthetic triggers used in probands. Succinylcholine was used in 76.3% (71 of 93) MH cases, either in combination with volatiles (71%, 66 of 93) or alone (5.4%, 5 of 93), whereas volatile agents were administered without succinylcholine in 23.7% (22 of 93) cases. Succinylcholine use did not differ significantly in male (77.4%, 48 of 62) *versus* female probands (73.3%, 22 of 30; $P = 0.667$).

MH Phenotype Severity

We used Clinical Grading Scale scores and MH onset time as quantitative indicators of MH phenotype severity. The median time to MH onset was 10 min (interquartile range 5 to 60) after exposure to trigger anesthetics, whereas the mean Clinical Grading Scale score was 47.9 (SD: 17.1). There was no significant association between the *RYR1* genotypes and either indicator of MH phenotype severity (table 3).

Both Clinical Grading Scale score and MH onset time positively correlated with age (Spearman's correlation coefficient 0.31, $P = 0.003$; and 0.39, $P = 0.001$, respectively), which implies that MH reactions in older patients were identified later and were more severe as per Clinical Grading Scale score (fig. 4).

Table 3. Proband's Age at MH Crisis and Clinical MH Indices as per *RYR1* Mutation

***RYR1* Mutation**	**Age (yr)**	**Onset t (min)**	**CGS**
p.Gly341Arg	23 [14–34]	25 [8–45]	55 ± 9
p.Arg614Cys	8 [6–32]	5 [2–5]	47 ± 21
p.Arg614Leu	12 [6–23]	2 [2–11]	46 ± 10
p.Thr2206Met	11 [5–25]	10 [5–79]	48 ± 16
p.Arg2336His	9.5 [7.75–11.25]	10 —	46 ± 11
p.Ala2350Thr	31.5 [23.75–39.25]	60 —	33
p.Gly2375Ala	41.5 [28.5–49.5]	33 [19–46]	50 ± 15
p.Gly2434Arg	6 [5.5–28.5]	12 [10–80]	46 ± 20
p.Arg2454His	13 [8.5–26]	98 [56–161]	45 ± 20
Overall	12 [6–32.5]	10 [5–60]	50 ± 17

Age and MH onset time are shown as median [interquartile range]; Clinical Grading Scale (CGS) score is expressed as mean ± SD. MH, malignant hyperthermia; *RYR1*, ryanodine receptor 1.

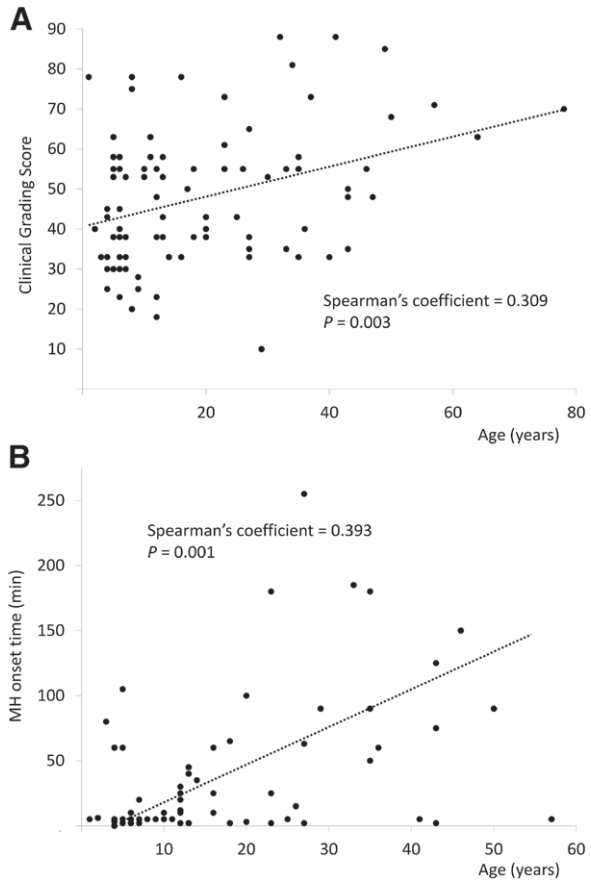

Fig. 4. Scatter plot showing a positive correlation between age and (*A*) the malignant hyperthermia (MH) Clinical Grading Score, or (*B*) the MH crisis onset time.

Discussion

This multicenter case–control study aimed to assess the penetrance of MH diagnostic *RYR1* mutations and examine factors that influence their expression. MH is a rare genetic syndrome with an incidence of 1:35,000 to 1:68,000 MH crises per all surgical discharges,[2,32] which makes it challenging for a single MH research center to amass sufficient data for a comprehensive penetrance study. Therefore, we combined clinical and genetic data on 125 European and Canadian MH families.

We quantified the penetrance of nine MH diagnostic mutations to be around 40%. We found that probands' likelihood to develop MH on exposure to triggers is higher compared with other *RYR1* mutation carriers. In fact, most probands in our sample developed MH during their first exposure to general anesthesia. We also found higher penetrance in males, despite similar exposures to triggers in both sexes.

Reduced penetrance is a phenomenon that blurs the distinction between genetically complex disorders and monogenic conditions with Mendelian inheritance, resulting from the interaction of multiple genetic and nongenetic factors that hamper establishing straightforward causation from known genotypes to specific phenotypes.[33] Factors influencing the penetrance of a genetic trait include the degree of dysfunction caused by a specific mutation(s), the modulating influence of additional variants on allelic expression, interindividual variations in gene expression, allele dosage causing homozygotes or compound-heterozygotes to have more severe phenotype, age- and sex-specific epigenetic changes such as genomic imprinting leading to the mutually exclusive expression of either the maternal or the paternal allele, and environmental influences such as the diet, alcohol intake, drugs, body habitus, and comorbid disease states. Any of these factors may either ameliorate or exacerbate the impact of the underlying genetic predisposition.[29]

Concerning MH, although a possible role of allele silencing in relation to other MH loci has not been explored yet, monoallelic silencing does not seem to affect the penetrance of MH-associated *RYR1* mutations,[34] and the role of allele-specific differences in expression levels of RyR1 transcript remains to be elucidated.[22]

Our study may lack the necessary power to achieve statistical significance regarding the variation in penetrance among the analyzed *RYR1* mutations. However this should not be interpreted as lack of differences in severity of phenotypes. Comparison of *in vivo* and *in vitro* in knock-in mouse models of MH demonstrate differences among the p.G2435R,[35] p.R163C,[36] and p.T4826I variants.[37]

In a large retrospective study by Carpenter *et al.*,[38] different *RYR1* genotypes in a group of MH probands were associated with magnitude of contracture in the *in vitro* contracture test and serum creatine kinase concentration, and both parameters were also associated with the clinical phenotype severity defined as MH onset time. The functional data from animal models alongside the human data from Carpenter *et al.* may suggest that the observed differences in severity of phenotype and penetrance among different variants may actually be real.

The Clinical Grading Scale scoring system was conceived to estimate the likelihood that an observed adverse anesthetic outcome was attributable to MH.[28] However, because of its *post hoc* nature, Clinical Grading Scale reliability depends on the availability of clinical data. It may underestimate the likelihood of MH in cases where the crisis is promptly recognized and treated. Despite these, we deemed it reasonable to use Clinical Grading Scale as quantitative indicator of MH phenotype severity because it rates the importance of clinical variables that appear during an MH event by assigning them a score. The positive correlation of both Clinical Grading Scale scores and time of MH onset with the patient's age observed in this study may suggest that diagnosis tends to be more delayed with increasing age.

We also explored the influence of nongenetic factors, such as sex and age, on MH penetrance. In our pool of probands, there were at least two males for every female, but there was a slightly greater proportion of women among relatives (71 of 133, 53.3%). A bias from sex imbalance would not arise if our study groups were matched according to sex, but then no effect estimate for sex could be derived.

Sex differences pervade the literature on MH, from the earliest epidemiologic reports[23,24] to the most recent survey demonstrating that the prevalence of MH in male patients doubles that of females.[2] It was also shown that more males than females test positive on the diagnostic contracture test for MH.[26] Sex-dependent susceptibility to MH is also present in mouse models.[37] Recently, male sex and body build subjectively assessed as muscular have been reported as independent predictors of MH susceptibility.[39] However, whether a larger muscle mass is associated with MH sex discrepancy warrants objective assessment. On the whole, the pathomechanism leading to sex differences in the penetrance of MH is still unknown, but epigenetic *RYR1* allele silencing has been ruled out as a cause of reduced penetrance of MH susceptibility in females.[34]

Age distribution in MH probands is known to be positively-skewed with younger people being most affected.[1,40] Several studies found that children aged 15 or younger comprised more than 50% of all reactions.[1,41,42] Although the majority of cases described here involved also children younger than 15 yr old, our probands' median age of 12 yr is lower than previously reported.[40] This possibly reflects the difference in composition of the investigated cohorts, because the former study included mostly adult patients whose MH status were confirmed by muscle biopsy and contracture testing.

The contrast between the low penetrance of some *RYR1* mutations and the high likelihood of probands to develop MH ($P_{\text{MH }probands}$) strengthens the notion of a multifactorial origin of MH, where genetic predisposition is necessary but not sufficient to unleash the MH syndrome. Although co-inheritance of other genetic factors should be taken into account, nongenetic influences, such as the anesthetic technique, the type of surgery, and patient's comorbidities, may play a pivotal role. In fact, we observed that in our series succinylcholine was used in 76% of MH cases (71% along with volatile anesthetics), which is far above the 10% average use in a typical North American hospital nowadays.[43] Because a number of our cases date back to the 1990s, this may reflect the bygone routine use of succinylcholine. Despite lacking relevant data about the use of succinylcholine in the relatives of our probands, the observed bias in the drug use in probands could be explained by the higher occurrence of MH during emergency surgery where succinylcholine is commonly used. Although ear, nose, and throat procedures seem to be the most common reason for surgery in the majority of MH cases in children,[2,44] orthopedic surgery and other emergent procedures are preponderant among adults.[29,41,45] Anecdotal reports of MH occurring during or shortly after emergent surgery for acute appendicitis are not rare in the medical literature.[46–54] It seems thus reasonable to suggest that a 10- to 20-fold increased risk of MH secondary to succinylcholine use[42,43] might not be solely attributable to the effects of the drug on the intracellular calcium dynamics of genetically predisposed patients, but also to a threshold shift induced by fever or a concomitant inflammatory process like sepsis or trauma, priming the onset of MH. We deem our observation worthy of further investigation.

There are limitations inherent to our study design. Data collection was retrospective and based on prevalent cases, carrying the risk of recall bias. Although the sample of patients was selected based on all available data, its composition may differ from that of the whole MH population because of participation and ascertainment bias (*e.g.*, identification of a causal mutation in a family preventing further exposures to MH triggers on its members, probands with weaker or abortive reactions remaining unrecognized, lower likelihood of asymptomatic relatives to be tested). We excluded patients with missing data and those with *RYR1* variants of unknown significance, which may have affected the overall power of the study. Because data from first- and second-degree relatives only were available for this study, penetrance in higher-order relatives remains an important investigation for the future.

In conclusion, the penetrance of nine MH-diagnostic *RYR1* mutations is incomplete and sex-dependent. Among genotype-positive subjects, probands have the highest risk of developing MH on exposure to trigger anesthetics. Young age, male sex, and the use of succinylcholine seem to be major nongenetic risk factors influencing expression of the *RYR1* genotypes conferring MH susceptibility in our cohort. Our quantitative evaluation of MH penetrance reinforces the notion that a previous uneventful anesthetic does not preclude the possibility of developing MH.

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Competing Interests

Dr. Riazi received a one-time honorarium from Norgine B.V. (Amsterdam, The Netherlands). The other authors declare no competing interests.

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